Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	720	sulfolobus or acidocaldarius	US-PGPUB; USPAT	OR	OFF	2004/02/26 14:37
L2	8584	$trehalose	US-PGPUB; USPAT	OR	OFF	2004/02/26 14:38
L3	157	non adj reducing adj saccharide$1	US-PGPUB; USPAT	OR	OFF	2004/02/26 14:39
L4	20	1 same (2 or 3)	US-PGPUB; USPAT	OR	OFF	2004/02/26 14:39

PGPUB-DOCUMENT-NUMBER: 20020197605

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197605 A1

TITLE: Novel Polynucleotides

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

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Mizoguchi, Hiroshi	Tokyo		JP	
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APPL-NO: 09/ 738626

DATE FILED: December 18, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	P. HEI 11-377484	1999JP-P. HEI 11-377484	December 16, 1999
JP	P. 2000-159162	2000JP-P. 2000-159162	April 7, 2000
JP	P. 2000-280988	2000JP-P. 2000-280988	August 3, 2000

US-CL-CURRENT: 435/6, 435/287.2 , 435/91.2

ABSTRACT:

Novel polynucleotides derived from microorganisms belonging to coryneform bacteria and fragments thereof, polypeptides encoded by the polynucleotides and fragments thereof, polynucleotide arrays comprising the polynucleotides and fragments thereof, recording media in which the nucleotide sequences of the polynucleotide and fragments thereof have been recorded which are readable in a computer, and use of them.

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Detail Description Table CWU - DETL (27):
sp:RIMM_MYCLE Mycobacterium leprae 52.3 72.1 172 16S rRNA processing protein MLCB250.34. rimM 2250 5750 2164390 2164737 348 pir:B71881 Helicobacter pylori J99 jhp0839 29.0 66.7 69 hpothetical protein 2251 5751 2165309 2164815 495 pir:C47154 Bacillus subtilis 168 rpsP 47.0 79.5 83 30S ribosomal protein S16 2252 5752 2165523 2166098 576 pir:T14151 Mus musculus inv 32.1 61.7 196 inversin 2253 5753 2166990 2166124 867 prf:2512328G Streptococcus agalactiae cylB 26.6 69.1 256 ABC transporter 2254 5754 2167865 2166990 876 prf:2220349C Pyrococcus horikoshii OT3 mtrA 35.5 63.8 318 ABC transporter 2255 5755 2169584 2167944 1641 sp:SR54_BACSU Bacillus subtilis 168 ffh 58.7 78.2 559 signal recognition particle protein 2256 5756 2170426

2171058 633 2257 5757 2171715 2172131 417 2258 5758 2172209 2172877 669
2259 5759 2175288 2173759 1530 sp:FTSY_ECOLI Escherichia coli K12 ftsY 37.0
66.1 505 cell division protein 2260 5760 2176046 2175888 159 2261 5761
2176402 2177103 702 2262 5762 2179502 2176110 3393 sp:AMYH_YEAST
Saccharomyces cerevisiae 22.4 46.2 1144 glucan 1,4-alpha-glucosidase or S288C
YIR019C sta1 glucoamylase S1/S2 precursor 2263 5763 2180918 2181880 963 2264
5764 2183092 2179628 3465 sp:Y06B_MYCTU Mycobacterium tuberculosis 48.3 72.6
1206 chromosome segregation protein H37Rv Rv2922c smc 2265 5765 2183391
2183110 282 sp:ACYP_MYCTU Mycobacterium tuberculosis 51.1 73.9 92
acylphosphatase H37Rv RV2922.1C 2266 5766 2185258 2183405 1854 2267 5767
2186208 2185351 858 sp:YFER_ECOLI Escherichia coli K12 yfeR 23.9 60.0 305
transcriptional regulator 2268 5768 2186299 2187129 831 pir:S72748
Mycobacterium leprae 39.3 73.5 257 hypothetical membrane protein MLCL581.28c
2269 5769 2187160 2187342 183 2270 5770 2187679 2187233 447 2271 5771
2188306 2187692 615 gp:DNINTREG_3 Dichelobacter nodosus gep 46.8 76.6 188
cation efflux system protein 2272 5772 2189170 2188313 858 sp:FPG_ECOLI
Escherichia coli K12 mutM or 36.1 66.7 285 formamidopyrimidine-DNA fpg
glycosylase 2273 5773 2189906 2189166 741 pir:B69693 Bacillus subtilis 168
rncS 40.3 76.5 221 ribonuclease III 2274 5774 2190439 2189906 534
sp:Y06F_MYCTU Mycobacterium tuberculosis 35.8 62.5 176 hypothetical protein
H37Rv Rv2926c 2275 5775 2191328 2190540 789 sp:Y06G_MYCTU Mycobacterium
tuberculosis 50.0 76.9 238 hypothetical protein H37Rv Rv2927c 2276 5776
2191522 2193165 1644 prf:2104260G Streptomyces verticillus 28.3 55.6 559
transport protein 2277 5777 2193165 2194694 1530 sp:CYDC_ECOLI Escherichia
coli K12 cydC 26.6 58.8 541 ABC transporter 2278 5778 2196883 2198004 1122
gp:SC9C7_2 Streptomyces coelicolor A3(2) 35.3 62.6 388 hypothetical protein
SC9C7.02 2279 5779 2198447 2198007 441 2280 5780 2198475 2199758 1284
pir:A72322 Thermotoga maritima MSB8 21.0 43.7 405 hypothetical protein TM0896
2281 5781 2199808 2201070 1263 sp:HIPO_CAMJE Campylobacter jejuni ATCC 32.9
64.3 353 peptidase 43431 hipO 2282 5782 2201408 2201073 336 pir:S38197
Arabidopsis thaliana SUC1 27.1 51.9 133 sucrose transport protein 2283 5783
2201584 2201450 135 2284 5784 2201869 2201594 276 2285 5785 2204541 2201992
2550 prf:2513410A Thermococcus litoralis malP 36.1 67.4 814 maltodextrin
phosphorylase / glycogen phosphorylase 2286 5786 2205490 2204591 900
sp:YFIE_BACSU Bacillus subtilis 168 yfiE 33.9 66.4 295 hypothetical protein
2287 5787 2208249 2207302 948 sp:LGT_STAAU Staphylococcus aureus FDA 485 31.4
65.5 264 prolipoprotein diacylglyceryl lgt transferase 2288 5788 2209167
2208367 801 sp:TRPG_EMENI Emericella nidulans trpC 29.6 62.1 169
indole-3-glycerol-phosphate synthase/anthranilate synthase component II 2289
5789 2209888 2209232 657 pir:H70556 Mycobacterium tuberculosis 29.4 58.8 228
hypothetical membrane protein H37Rv Rv1610 2290 5790 2210273 2209920 354
sp:HIS3_RHOSH Rhodobacter sphaeroides ATCC 52.8 79.8 89 phosphoribosyl-AMP
cyclohydrolase 17023 hisI 2291 5791 2211046 2210273 774 sp:HIS6_CORG
Corynebacterium glutamicum 97.3 97.7 258 cyclase AS019 hisF 2292 5792
2211875 2211051 825 prf:2419176B Corynebacterium glutamicum 94.0 94.0 241
inositol monophosphate AS019 impA phosphatase 2293 5793 2212619 2211882 738
gp:AF051846_1 Corynebacterium glutamicum 95.9 97.6 245
phosphoribosylformimino-5- AS019 hisA aminoimidazole carboxamide ribotide
isomerase 2294 5794 2213273 2212641 633 gp:AF060558_1 Corynebacterium
glutamicum 86.7 92.4 210 glutamine amidotransferase AS019 hisH 2295 5795
2215586 2214321 1266 sp:CMLR_STRLI Streptomyces lividans 66 cmlR 25.6 54.0 402
chloramphenicol resistance protein or transmembrane transport protein 2296
5796 2215863 2215639 225 2297 5797 2216474 2215869 606 sp:HIS7_STRCO
Streptomyces coelicolor A3(2) 52.5 81.8 198 imidazoleglycerol-phosphate hisB
dehydratase 2298 5798 2217591 2216494 1098 sp:HIS8_STRCO Streptomyces
coelicolor A3(2) 57.2 79.3 362 histidinol-phosphate hisC aminotransferase
2299 5799 2218925 2217600 1326 sp:HISX_MYCSM Mycobacterium smegmatis 63.8 85.7
439 histidinol dehydrogenase ATCC 607 hisD 2300 5800 2219159 2220358 1200
gp:SPBC215_13 Schizosaccharomyces pombe 27.2 54.4 342 serine-rich secreted

protein SPBC215.13 2301 5801 2221109 2220459 651 2302 5802 2221611 2221919 309 2303 5803 2221828 2221187 642 prf:2321269A Leishmania donovani SAcP-1 29.4 59.7 211 histidine secretory acid phosphatase 2304 5804 2221958 2222518 561 pir:RPECR1 Escherichia coli plasmid RP1 28.9 60.8 204 tet repressor protein tetR 2305 5805 2222528 2225035 2508 prf:2307203B Sulfolobus acidocaldarius treX 47.4 75.5 722 glycogen debranching enzyme 2306 5806 2225149 2225949 801 pir:E70572 Mycobacterium tuberculosis 50.0 76.0 258 hypothetical protein H37Rv Rv2622 2307 5807 2226763 2225990 774 gp:SC2G5_27 Streptomyces coelicolor A3(2) 29.9 55.2 268 oxidoreductase SC2G5.27c gip 2308 5808 2227779 2226769 1011 prf:2503399A Sinorhizobium meliloti idhA 35.0 60.9 343 myo-inositol 2-dehydrogenase 2309 5809 2227906 2228901 996 sp:GALR_ECOLI Escherichia coli K12 galR 30.4 64.4 329 galactitol utilization operon repressor 2310 5810 2229896 2229099 798 sp:FHUC_BACSU Bacillus subtilis 168 fhuC 32.9 68.3 246 ferrichrome transport ATP-binding protein or ferrichrome ABC transporter 2311 5811 2230937 2229900 1038 prf:2423441E Vibrio cholerae hutC 36.8 71.1 332 hemin permease 2312 5812 2231294 2230947 348 pir:G70046 Bacillus subtilis 168 yvrC 30.1 68.0 103 iron-binding protein 2313 5813 2231932 2231339 594 pir:G70046 Bacillus subtilis 168 yvrC 34.6 67.6 182 iron-binding protein 2314 5814 2232456 2232016 441 sp:YTFH_ECOLI Escherichia coli K12 ytfH 38.1 73.5 113 hypothetical protein 2315 5815 2232928 2234070 1143 gp:SCI8_12 Streptomyces coelicolor A3(2) 23.4 50.1 355 DNA polymerase III epsilon chain SCI8.12 2316 5816 2234158 2234763 606 2317 5817 2234852 2237284 2433 pir:S65769 Arthrobacter sp. Q36 treY 42.0 68.6 814 maltooligosyl trehalose synthase 2318 5818 2237331 2238353 1023 gp:AE002006_4 Deinococcus radiodurans 27.6 52.8 322 hypothetical protein DR1631 2319 5819 2239092 2238694 399 2320 5820 2240042 2239845 198 2321 5821 2240246 2240058 189 2322 5822 2240563 2239508 1056 2323 5823 2240681 2241724 1044 sp:LXA1_PHOLU Photorhabdus luminescens 20.5 54.4 375 alkanal monooxygenase alpha chain ATCC 29999 luxA 2324 5824 2242115 2241738 378 gp:SC7H2_5 Streptomyces coelicolor A3(2) 58.3 79.2 120 hypothetical protein SC7H2.05 2325 5825 2242359 2242129 231 2326 5826 2243035 2244819 1785 pir:S65770 Arthrobacter sp. Q36 treZ 46.3 72.4 568 maltooligosyltrehalose trehalohydrolase 2327 5827 2243043 2242393 651 sp:YVYE_BACSU Bacillus subtilis 168 36.5 72.4 214 hypothetical protein 2328 5828 2246171 2244864 1308 sp:THD1_CORGL Corynebacterium glutamicum 99.3 99.3 436 threonine dehydratase ATCC 13032 ilvA 2329 5829 2246386 2246892 507 2330 5830 2246450 2246295 156 2331 5831 2248208 2247006 1203 pir:S57636 Catharanthus roseus metE 22.7 49.6 415 Corynebacterium glutamicumASO19 2332 5832 2251939 2248358 3582 prf:2508371A Streptomyces coelicolor A3(2) 53.3 80.5 1183 DNA polymerase III dnaE 2333 5833 2252017 2252856 840 sp:RARD_ECOLI Escherichia coli K12 rarD 37.6 73.8 279 chloramphenicol sensitive protein 2334 5834 2253192 2253659 468 sp:HISJ_CAMJE Campylobacter jejuni DZ72 hisJ 21.5 55.7 149 histidine-binding protein precursor 2335 5835 2253725 2254642 918 pir:D69548 Archaeoglobus fulgidus AF2388 22.7 64.7 198 hypothetical membrane protein 2336 5836 2255558 2254683 876 sp:GS39_BACSU Bacillus subtilis 168 ydaD 48.2 80.0 280 short chain dehydrogenase or general stress protein 2337 5837 2257024 2255738 1287 sp:DCDA_PSEAE Pseudomonas aeruginosa lysA 22.9 47.6 445 diaminopimelate (DAP) decarboxylase 2338 5838 2259312 2258362 951 sp:CYSM_ALCEU Alcaligenes eutrophus CH34 32.8 64.3 314 cysteine synthase cysM 2339 5839 2259999 2259421 579 2340 5840 2260931 2260002 930 sp:RLUD_ECOLI Escherichia coli K12 rluD 36.5 61.0 326 ribosomal large subunit pseudouridine synthase D 2341 5841 2261467 2260934 534 sp:LSPA_PSEFL Pseudomonas fluorescens NCIB 33.8 61.7 154 lipoprotein signal peptidase 10586 lspA 2342 5842 2261688 2262689 1002

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

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Hendriksen, Hanne Vang	Holte		DK	
Svendsen, Allan	Birkerod		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	
Nielsen, Rudy Illum	Farum		DK	

APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718

parent continuation-of 09632392 20000804 US GRANTED

parent-patent 6303346 US

child 09632392 20000804 US

parent continuation-of 09499531 20000210 US GRANTED

parent-patent 6136571 US

child 09499531 20000210 US

parent continuation-of 09198672 19981123 US GRANTED

parent-patent 6129788 US

child 09198672 19981123 US

parent continuation-in-part-of 09107657 19980630 US ABANDONED

child 09107657 19980630 US

parent continuation-in-part-of 08979673 19971126 US ABANDONED

US-CL-CURRENT: 435/96

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more

enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.
In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

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Detail Description Paragraph - DETX (49):
[0104] Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae Sulfolobus solfataricus KM1.

PGPUB-DOCUMENT-NUMBER: 20020102696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102696 A1

TITLE: RECOMBINANT THERMOSTABLE ENZYME WHICH FORMS NON-REDUCING SACCHARIDE FROM REDUCING AMYLACEOUS SACCHARIDE

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

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KUBOTA, MICHIO	OKYAMA		JP	
SUGIMOTO, TOSHIYUKI	OKAYAMA		JP	

APPL-NO: 09/ 419305

DATE FILED: October 15, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09419305 A1 19991015

parent continuation-of 08505448 19950721 US PATENTED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	190183/1994	1994JP-190183/1994	July 21, 1994
JP	189706/1995	1995JP-189706/1995	July 4, 1995

US-CL-CURRENT: 435/200, 435/183

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

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Summary of Invention Paragraph - BSTX (9):

[0008] In view of the foregoing, the present inventors screened thermostable enzyme with such a novel enzyme activity and have found that enzymes produced from microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a

temperature exceeding 55.degree. C., and they efficiently produce such non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides. These micro-organisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce non-reducing saccharides having a trehalose structure as an end unit.

Detail Description Paragraph - DETX (8):

[0042] The present invention has been accomplished based on the finding of a novel enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

US-PAT-NO: 6391595

DOCUMENT-IDENTIFIER: US 6391595 B1

TITLE: Transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same

DATE-ISSUED: May 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kato; Masaru	Takasaki	N/A	N/A	JP
Miura; Yutaka	Takasaki	N/A	N/A	JP

APPL-NO: 09/ 298924

DATE FILED: April 26, 1999

PARENT-CASE:

This application is a Divisional of application Ser. No. 08/750,569, filed Feb. 24, 1997, which is a national stage of PCT/JP95/01189 filed Jun. 14, 1995.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-133354	June 15, 1994
JP	6-194223	August 18, 1994
JP	6-290394	October 31, 1994
JP	6-286917	November 21, 1994
JP	6-311185	November 21, 1994
JP	7-120673	April 21, 1995

US-CL-CURRENT: 435/100, 435/183 , 435/194 , 435/200 , 435/91.53 , 435/97

ABSTRACT:

The invention provides a novel transferase that acts on a saccharide, as a substrate, composed of at least three sugar units wherein at least three glucose residues on the reducing end are linked .alpha.-1,4 so as to transfer the .alpha.-1,4 lingages to a .alpha.-1,.alpha.-1 linkages; a process for producing the transferase; a gene coding for the same; and a process for producing an oligosaccharide by using the same. Also provided are a novel amylase that has a principal activity of acting on a saccharide, as a substrate, composed of at least three sugar units wherein at least three sugar units on the reducing end side are glucose units and the linkage between the first and the second glucose units is .alpha.-1,.alpha.-1 while the linkage between the second and the third glucose units is .alpha.-1,4 so as to liberate .alpha.,.alpha.-trehalose by hydrolyzing the .alpha.-1,4 linkage and another activity of hydrolyzing the .alpha.-1,4 linkage within the molecular chain of the substrate and that liberates disaccharides and/or monosaccharides as the principal final products; a process for producing the amylase; a gene coding for the same; and a process for producing .alpha.,.alpha.-trehalose by using a combination of the transferase and the amylase.

38 Claims, 51 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 44

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Brief Summary Text - BSTX (8):

II. The present invention relates to a novel amylase which acts on a substrate saccharide, the saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end are glucose residues, so as to liberate principally monosaccharides and/or disaccharides by hydrolyzing the substrate from the reducing end; and a process for producing the amylase. More particularly, the present invention relates to a novel amylase which has an principal activity of acting on a substrate saccharide, the substrate saccharide being composed of at least three sugar units wherein at least three sugar units from the reducing end side are glucose residues and the linkage between the first and the second glucose residues from the reducing end side is .alpha.-1,.alpha.-1 while the linkage between the second and the third glucose residues from the reducing end side is .alpha.-1,4, so as to liberate .alpha.,.alpha.-trehalose by hydrolyzing the .alpha.-1,4 linkage between the second and the third glucose residues; and a process for producing the amylase. The novel amylase also has another activity of endotype-hydrolyzing one or more .alpha.-1,4 linkages within the molecular chain of the substrate, and can be produced by bacteria belonging to the genus Sulfolobus. This enzyme is available for the starch sugar industry, textile industry, food industry, and the like.

Brief Summary Text - BSTX (18):

Recently, Lama, et al. found that a cell extract from the Sulfolobus solfataricus strain MT-4 (DSM 5833), a species of archaebacteria, has a thermostable starch-hydrolyzing activity [Biotech. Forum. Eur. 8, 4, 2-1 (1991)]. They further reported that the activity is also of producing trehalose and glucose from starch. The above-mentioned report, however, does not at all refer to the existence of trehaloseoligosaccharides such as glucosyltrehalose and maltooligosyltrehalose. Moreover, no investigation in archaebacteria other than the above-mentioned strain has been attempted.

Brief Summary Text - BSTX (22):

Inventors earnestly investigated the trehalose-producing activity of archaebacteria and found that glucosyltrehalose can be produced from maltotriose as a substrate by cell extracts from various archaebacteria such as those belonging to the order Sulfolobales, and more specifically, the genera Sulfolobus, Acidianus, and others. Here, though production of trehalose and glucose was confirmed using an activity-measuring method described by Lama, et al. in which the substrate is starch, Inventors found that detection of trehaloseoligosaccha-rides such as glucosyltrehalose is extremely difficult. Also, Inventors found that the trehalose-producing activity as found by Lama, et al. disappears during the step for purification of cell extracts from archaebacteria. Consequently, the inventors recognized that the purification and characterization of the enzymes themselves which have such activities were substantially impossible.

Brief Summary Text - BSTX (33):

being sulfur bacteria: being cocci having irregular form, and a diameter of 0.6-2 .mu.m. Accordingly, if an archaebacterium belonging to the genus

Sulfolobus produces an amylase, the amylase is expected to be also thermo-stable. Lama, et al.found that a thermostable starch-hydrolyzing activity exists in a cell extract from the Sulfolobus solfataricus strain MT-4 (DSM 5833) [Biotech. Forum. Eur. 8, 4, 2-1 (1991)]. This article reported that .alpha.,.alpha.-trehalose and glucose can be produced from starch by this activity. However, purification of the active substance was performed only partially, and the true substance exhibiting the activity has not yet been identified. In addition, the enzymatic characteristics of the activity has not been clarified at all. The Inventors' investigations, the details of which will be described below, revealed that the active substance derived from the above-mentioned bacterial strain and allowed to act on starch by Lama, et al. was a mixture containing a plurality of enzymes, and that .alpha.,.alpha.-trehalose and glucose are the final products obtained by using the mixture.

Brief Summary Text - BSTX (40):

As described above, .alpha.,.alpha.-trehalose was found widely in nature, and the existence of it in archaebacteria was also confirmed (System. Appl. Microbiol. 10, 215, 1988). Specifically, as mentioned above, Lama, et al. found that a thermostable starch-hydrolyzing activity exists in a cell extract from an archaebacterium species, the Sulfolobus solfataricus strain MT-4 (DSM 5833), and confirmed the existence of .alpha.,.alpha.-trehalose in the hydrolyzed product [Biotech. Forum. Eur. 8, 4, 2-1 (1991), cited before]. This article reported that the activity was of producing .alpha.,.alpha.-trehalose and glucose from starch. The article, however, actually reported only an example in which the substrate was 0.33% soluble starch, the amount of .alpha.,.alpha.-trehalose produced thereby was extremely small, and besides, the ratio of produced .alpha.,.alpha.-trehalose to produced glucose was 1:2. Accordingly, an isolation process is necessary to remove glucose which is produced in a large amount as a by-product, and the purpose of establishing a process for mass-producing .alpha.,.alpha.-trehalose cannot be achieved at all.

Brief Summary Text - BSTX (45):

Inventors energetically investigated starch-hydrolyzing activity derived from archaebacteria. As a result, Inventors found that a thermostable starch-hydrolyzing activity exists in cell extracts from various archaebacteria belonging to the order Sulfolobales, and more specifically, the genus Sulfolobus. The saccharides produced by hydrolysis of starch were found to be glucose and .alpha.,.alpha.-trehalose, similar to the description in the article by Lama, et al. Inventors then examined extracts from various bacterial strains for characteristics of the starch-hydrolyzing activity. As a result, Inventors found that the enzymes produced by those strains are mixtures of enzymes comprising various endotype or exotype amylases such as liquefying amylase and glucoamylase, and transferase, in view of enzymatic activity such as starch-hydrolyzing activity and .alpha.,.alpha.-trehalose-producing activity. In addition, such enzymatic activities were found to be attributed to synergism by activities of these mixed enzymes. Further, when the activity-measuring method proposed by Lama, et al. is employed in purification of each enzyme, in which the index is decrement of blue color derived from iodo-starch reaction, the purification of each enzyme having such an activity resulted in a low yield on the whole, and such purification procedure was found to be very difficult. These events may be attributed to low sensitivity and low quantifying ability of the activity-measuring method. Moreover, the Inventors' strict examination revealed that purification and isolation could not be accomplished at all, in terms of protein, by the partial-purification method described in the article by Lama, et al.

Drawing Description Text - DRTX (11):
FIG. 10 is a graph showing the results of an analysis by TSK-gel Amide-80 HPLC, performed on the reaction product derived from maltotriosyltrehalose subjected to reaction with the crude enzyme solution which is obtained in Example II-1 from the Sulfolobus solfataricus strain KM1.

Drawing Description Text - DRTX (19):
FIG. 18 is a graph showing the results of an analysis by TSK-gel Amide-80 HPLC, performed on the reaction product derived from maltotriosyltrehalose subjected to reaction with the present amylase which is obtained in Example II-2 from the Sulfolobus solfataricus strain KM1.

Drawing Description Text - DRTX (20):
FIG. 19 is a graph showing the results of an analysis by TSK-gel Amide-80 HPLC, performed on the reaction product derived from maltopentaosyltrehalose subjected to reaction with the present amylase which is obtained in Example II-2 from the Sulfolobus solfataricus strain KM1.

Drawing Description Text - DRTX (23):
FIG. 22 is a graph showing time-course change in radioactivity of the reaction product derived from radiolabeled maltotriosyltrehalose subjected to reaction with the present amylase which is obtained in Example II-2 from the Sulfolobus solfataricus strain KM1.

Detailed Description Text - DETX (99):
The transferase of the present invention which is described in detail in the above-described item "I. Novel Transferase" can be used for production of .alpha.,.alpha.-trehalose according to the present invention. Specifically, examples of such a transferase may include transferases derived from the Sulfolobus solfataricus strain ATCC 35091 (DSM 1616), the Sulfolobus solfataricus strain DSM 5833, the Sulfolobus solfataricus strain KM1, the Sulfolobus acidocaldarius strain ATCC 33909 (DSM 639), and the Acidianus brierleyi strain DSM 1651.

Detailed Description Text - DETX (276):
The trehaloseoligosaccharide-hydrolyzing activity is expressed with such a unit as 1 Unit equals the activity of liberating 1 .mu.mol of .alpha.,.alpha.-trehalose per hour from maltotriosyltrehalose. Incidentally, in Table 11, the activity is expressed in terms of units per one gram of bacterial cell. Here, maltotriosyltrehalose was prepared as follows: The purified transferase derived from the Sulfolobus solfataricus strain KM1 was added to a 10% maltopentaose solution containing 50 mM of acetic acid (pH 5.5) so that the concentration of the transferase would be 10 Units/ml; the mixture thus obtained was subjected to a reaction at 60.degree. C. for 24 hours; and the resultant was subjected to the above TSK-gel Amide-80 HPLC column to obtain maltotriosyltrehalose. As to the activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as equalling the activity of producing 1 .mu.mol of glucosyltrehalose per hour at 60.degree. C. and pH 5.5 when maltotriose is used as the substrate.

Detailed Description Text - DETX (287):

From the above results, the cell extract of a bacterial strain belonging to the genus Sulfolobus was found to have an activity of hydrolyzing trehaloseoligosaccharides to liberate .alpha.,.alpha.-trehalose, and an activity of hydrolyzing starch to liberate principally monosaccharides or disaccharides.

Detailed Description Text - DETX (351):

Uridinediphosphoglucose [glucose-6-.sup.3 H] and maltotetraose were put into a reaction with glycogen synthase (derived from rabbit skeletal muscle, G-2259 manufactured by Sigma Co.) to synthesize maltopentaose, of which the glucose residue of the non-reducing end was radiolabeled with .sup.3 H, and the maltopentaose was isolated and purified. To 10 mM of this maltopentaose radiolabeled with .sup.3 H as a substrate, 10 Units/ml (in terms of the enzymatic activity when maltotriose is used as the substrate) of the purified transferase derived from the Sulfolobus solfataricus strain KM1 was added and put into a reaction at 60.degree. C. for 3 hours. Maltotriosyltrehalose, of which the glucose residue of the non-reducing end was radiolabeled with .sup.3 H, was synthesized thereby, and the product was isolated and purified. [Incidentally, it was confirmed by the following procedure that the glucose residue of the non-reducing end had been radiolabeled: The above product was completely decomposed into glucose and .alpha.,.alpha.-trehalose by glucoamylase (derived from Rhizopus, manufactured by Seikagaku Kougyou Co.); the resultants were sampled by thin-layer chromatography, and their radioactivities were measured by a liquid scintillation counter; as a result, radioactivity was not observed in the .alpha.,.alpha.-trehalose fraction but in the glucose fraction.]

Detailed Description Text - DETX (374):

As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (381):

Production of .alpha.,.alpha.-trehalose utilizing the synergism between enzymes was attempted by adding the enzymes having concentrations listed in Table 19, respectively, to a substrate (final concentration: 10%). Specifically, the enzymes were the present purified enzyme obtained in Example II-2, and the purified transferase derived from the Sulfolobus solfataricus strain KM1; the substrate was a soluble starch which had been pre-treated under the conditions of 40.degree. C. for 1 hour with 25 Units/ml of pullulanase (manufactured by Wako pure chemical Co.) derived from Klebsiella pneumoniae; and the reaction was performed under the conditions of 60.degree. C. at pH 5.5 for 100 hours, approximately. After the non-reacted substrate was hydrolyzed with glucoamylase, the reaction mixture was analyzed by the TSK-gel Amide-80 HPLC analyzing method described in Example II-1 to examine the yield of the produced .alpha.,.alpha.-trehalose.

Detailed Description Text - DETX (383):

As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (395):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (401):
Production of .alpha.,.alpha.-trehalose utilizing the synergism between enzymes was attempted by adding the enzymes having concentrations listed in Table 21, respectively, to a substrate (final concentration: 10%). Specifically, the enzymes were the present purified enzyme obtained in Example II-2, and the purified transferase derived from the Sulfolobus solfataricus strain KM1; the substrate was Amylose DP-17 (manufactured by Hayashibara Biochemical Co.); and the reaction was performed under the conditions of 60.degree. C. at pH 5.5 for 100 hours, approximately. After the non-reacted substrate was hydrolyzed with glucoamylase, the reaction mixture was analyzed by the TSK-gel Amide-80 HPLC analyzing method described in Example II-1 to examine the yield of the produced .alpha.,.alpha.-trehalose.

Detailed Description Text - DETX (403):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (408):
Production of .alpha.,.alpha.-trehalose utilizing the synergism between enzymes was attempted by adding the enzymes having concentrations listed in Table 22, respectively, to a substrate, the final concentration of which would be adjusted at 5%, 10%, 20% or 30%. Specifically, the enzymes were the present purified enzyme obtained in Example II-2, and the purified transferase derived from the Sulfolobus solfataricus strain KM1; the substrate was soluble starch; and the reaction was performed under the conditions of 60.degree. C. at pH 5.5 for 100 hours, approximately. During the reaction, from 0 hours to 96 hours after the start, a treatment at 40.degree. C. for 1 hour with the addition of pullulanase (a product derived from Klebsiella pneumoniae, manufactured by Wako pure chemical Co.) so as to be 5 Units/ml was performed every 12 hours, namely, totaling 9 times inclusive of the treatment at 0 hours.

Detailed Description Text - DETX (411):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (424):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (436):
As to activity of the purified transferase derived from the Sulfolobus

solfataricus strain KM1, Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (449):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (463):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (468):
Production of .alpha.,.alpha.-Trehalose from Soluble Starch with a Treatment Using a Debranching Enzyme Derived from the Sulfolobus solfataricus Strain KM1

Detailed Description Text - DETX (475):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (478):
As is obvious from the results shown in the tables, the yield can be improved by only one addition of the debranching enzyme derived from the Sulfolobus solfataricus strain KM1 during the reaction, similar to pullulanase (Debranching Enzyme Amano, a product derived from Bacillus sp.). The yield of .alpha.,.alpha.-trehalose reached to 69.8%.

Detailed Description Text - DETX (483):
As to activity of the purified transferase derived from the Sulfolobus solfataricus strain KM1, 1 Unit is defined as the enzymatic activity of producing 1 .mu.mol of glucosyltrehalose per hour at pH 5.5 and 60.degree. C. from maltotriose assigned for the substrate.

Detailed Description Text - DETX (501):
Incidentally, in this purification procedure, detection of the objective debranching enzyme was performed by mixing the sample solution with 2 Units/ml of the purified amylase and 32 Units/ml of the purified transferase derived from the Sulfolobus solfataricus strain KM1, and by putting the mixture into a reaction at 60.degree. C. and pH 5.5, wherein the index was the activity of achieving a higher yield of .alpha.,.alpha.-trehalose in comparison with the reaction without the sample solution.

Detailed Description Text - DETX (513):
As is obvious from the above results, the present debranching enzyme can 1) generate reducing ends in pullulan and various kinds of starch; 2) increase the

coloring degree in the iodo-starch reaction; 3) produce maltotriose from pullulan; and further, 4) as shown in Example II-14, markedly increase the yield of .alpha.,.alpha.-trehalose from soluble starch used as a substrate when the present debranching enzyme is put into the reaction with the purified amylase and transferase derived from the Sulfolobus solfataricus strain KM1, as compared with the reaction without the addition of the present debranching enzyme. As a consequence of these facts, the present enzyme is recognized as hydrolyzing .alpha.-1,6 linkages in starch or pullulan.

Detailed Description Text - DETX (739):
The yield of .alpha.,.alpha.-trehalose was 67% when 50 Units/ml of pullulanase was added. This value suggests that the recombinant novel amylase can bring about almost the same yield as the purified novel amylase derived from the Sulfolobus solfataricus strain KM1 can under the above reaction condition.

Detailed Description Paragraph Table - DETL (2):
TABLE 2 Sulfolobus Sulfolobus Sulfolobus Physicochemical solfataricus solfataricus acidocaldarius properties KM1 DSM5833 ATCC33909 (1) Enzyme action and Acts of glucose polymers composed of more than Substrate specificity maltotriose, so as to hydrolyze by endo-type and liberates principally monosaccharide or disaccharide from the reducing end. Especially liberates .alpha.,.alpha.-trehalose from trehaloseoligo- saccharide wherein the linkage between two glucoses from the reducing end side is .alpha.-1,.alpha.-1 while the other linkages are .alpha.-1,4. (2) Optimum pH 4.5-5.5 4.5-5.5 5.0-5.5 (3) pH Stability 3.5-10.0 3.0-12.0 4.0-13.0 (4) Optimum 70-85.degree. C. 70-85.degree. C. 60-80.degree. C. temperature (5) Thermal stability 85.degree. C., 6 hr 85.degree. C., 6 hr 80.degree. C., 6 hr 100% remained 100% remained 100% remained (6) Molecular weight SDS-PAGE 61000 62000 64000 (7) Isoelectric point 4.8 4.3 5.4 (8) Inhibitor 5 mM CuSO.sub.4 5 mM CuSO.sub.4 5 mM CuSO.sub.4 100% inhibited 100% inhibited 100% inhibited

Detailed Description Paragraph Table - DETL (11):
TABLE 11 Enzyme activity (units/g-cell) Hydrolyzing activity Hydrolyzing activity of trehalose Strain of starch oligosaccharide Sulfolobus solfataricus ATCC 35091 13.3 118.0 DSM 5354 13.3 116.8 DSM 5833 8.4 94.9 KM1 13.4 293.2 Sulfolobus acidocaldarius ATCC 33909 12.5 161.8 Sulfolobus shibatae DSM 5389 11.2 281.2

Claims Text - CLTX (24):
24. A process for producing .alpha.,.alpha.-trehalose, comprising: (a) subjecting a glucide raw material to endotype-hydrolysis that is catalyzed by an amylase enzyme derived from the genus Sulfolobus, under conditions comprising a temperature range of about 50.degree. C. to about 80.degree. C. and a pH range of about 3.5 to about 8, to produce a substrate selected from amylose and maltooligosaccharide; (b) transforming, into an .alpha.-1, .alpha.-1 linkage, the first .alpha.-1,4 linkage from the reducing end of said substrate, wherein said transformation is catalyzed by a transferase enzyme selected from the group consisting of a transferase derived from the genus Sulfolobus and a transferase derived from the genus Acidianus, and (c) hydrolyzing the .alpha.-1,4 linkage between the second and third glucose residues from the reducing end of the resultant substrate, to liberate .alpha.,.alpha.-trehalose therefrom, wherein said hydrolyzing comprises exposing said transformed substrate to an amylase enzyme that is derived from the genus Sulfolobus and that is present at a concentration of at least 1.5

units/ml.

US-PAT-NO: 6346394

DOCUMENT-IDENTIFIER: US 6346394 B1

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

DATE-ISSUED: February 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitsuzumi; Hitoshi	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP

APPL-NO: 09/ 055210

DATE FILED: April 6, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 08/798,269 filed Feb. 11, 1997, which is a division of application Ser. No. 08/505,377, filed Jul. 21, 1995, U.S. Pat. No. 5,856,146 the entire contents of both applications are herein incorporated by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190180	July 21, 1994
JP	7-109128	April 11, 1995
JP	7-189760	July 4, 1995

US-CL-CURRENT: 435/69.1, 435/183 , 435/200 , 435/252.33 , 435/320.1 , 435/69.2 , 435/71.1 , 435/71.2 , 435/97 , 536/23.1 , 536/23.2 , 536/23.7

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 54,000-64,000 daltons and a pI of about 5.6-6.6, and releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. The enzyme has a satisfactorily-high thermostability, i.e. it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of trehalose on an industrial scale and in a satisfactorily-high yield.

17 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (9):

In view of the foregoing, the present inventors screened thermostable enzyme with a satisfactory activity and have found that enzymes produced from microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently release trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. These microorganisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce trehalose from those non-reducing saccharides.

Detailed Description Text - DETX (8):

The present invention has been made based on the finding of a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

US-PAT-NO: 6303346

DOCUMENT-IDENTIFIER: US 6303346 B1

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 632392

DATE FILED: August 4, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/499,531 filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. Ser. No. 09/198,672 filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. Ser. No. 09/107,657 filed on Jun. 30, 1998, abandoned, which is a continuation-in-part of U.S. Ser. No. 08/979,673 filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 435/96, 127/40 , 127/55 , 435/105 , 435/98 , 435/99

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1,5,8

Number of Drawing Sheets: 5

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Detailed Description Text - DETX (49):

Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae Sulfolobus solfataricus KM1.

US-PAT-NO: 6150153

DOCUMENT-IDENTIFIER: US 6150153 A

TITLE: Thermostable trehalose-releasing enzyme

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ikegami; Shouji	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP
Miyake; Toshio	Okayama	N/A	N/A	JP

APPL-NO: 08/ 888158

DATE FILED: July 3, 1997

PARENT-CASE:

This is a continuation of parent application Ser. No. 08/485,132 filed Jun. 7, 1995, now U.S. Pat. No. 5,723,327.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-166126	June 25, 1994
JP	7-109130	April 11, 1995

US-CL-CURRENT: 435/252.1, 435/200 , 435/201 , 435/822

ABSTRACT:

Disclosed are novel thermostable trehalose-releasing enzyme, and its preparations and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of hydrolyzing at a temperature of over 55.degree. C. the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Trehalose and compositions containing the same are extensively useful in food products, cosmetics and pharmaceuticals.

1 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Abstract Text - ABTX (1):

Disclosed are novel thermostable trehalose-releasing enzyme, and its preparations and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of

hydrolyzing at a temperature of over 55.degree. C. the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Trehalose and compositions containing the same are extensively useful in food products, cosmetics and pharmaceuticals.

Brief Summary Text - BSTX (9):

On the other hand, since a thermostable enzyme can maintain its activity at a relatively-high temperature, contamination during the enzymatic reaction is less of a concern and the retrogradation of partial starch hydrolysates is scarcely caused. As a source of thermostable enzymes, thermophilic microorganisms can be generally considered. Regarding a preparation of trehalose using thermophilic microorganisms, as described in Biotechnology Letters, Vol.12, pp.431-432 (1990) and Biotech Forum Europe, Vol.8, pp.201-203 (1991), it was reported that the partially purified enzyme preparation obtainable from the cell and cell extract of Sulfolobus solfataricus (ATCC 49155) forms glucose and trehalose when allowed to act on substrate such as amylose and soluble starch. A purification of such as an enzyme preparation can not be completed, however, as the physicochemical properties of the enzyme thus prepared are not sufficiently indicated and the action of the enzyme has not been clarified, and only a preparation of trehalose is indicated. Thus, there has been a in great demand to establish a novel preparation of trehalose by utilizing a thermostable enzyme capable of acting at a temperature of over 55.degree. C.

Brief Summary Text - BSTX (13):

As a result, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, these as disclosed in Japanese Patent Application No.166,011/94, produce a thermostable non-reducing saccharide-forming enzyme and also a novel thermostable trehalose-releasing enzyme which are capable of acting at a temperature of over 55.degree. C., and found that the objective preparation of trehalose at a temperature of over 55.degree. C. is readily conducted by allowing the thermostable non-reducing saccharide enzyme together with this novel thermostable trehalose-releasing enzyme to act on reducing partial starch hydrolysates. The present inventors also found that trehalose is readily preparable by allowing the thermostable non-reducing saccharide-forming enzyme together with the novel thermostable trehalose-releasing enzyme to act on reducing partial starch hydrolysates and subjecting to the action of glucoamylase or .alpha.-glucosidase to obtain reacted solutions containing trehalose with a relatively-high purity. Thus, the present inventors accomplished this invention.

Detailed Description Text - DETX (4):

Now, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, are capable of producing a novel thermostable trehalose-releasing enzyme.

Detailed Description Text - DETX (5):

In addition to the above-mentioned microorganisms, other strains of the genus Sulfolobus and their mutants can be arbitrarily used in the present invention as long as they produce a thermostable trehalose-releasing enzyme which specifically hydrolyses the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose

structure as an end unit and having a degree of glucose polymerization of 3 or higher.

Detailed Description Text - DETX (27):
As non-reducing saccharide-forming enzymes which forms non-reducing saccharides having a trehalose structure and having a degree of glucose polymerization of 3 or higher when allowed to act on reducing partial starch hydrolysates, those derived from Rhizobium sp. M-11 or Arthrobactor sp. Q36 as disclosed in Japanese Patent Application No.349,216/93, can be used, however, in case that an enzymatic reaction proceeds at a temperature of over 55.degree. C., the thermostable non-reducing saccharide-forming enzyme which belongs to the group of the genus Sulfolobus, disclosed in Japanese Patent Application No.166,011/94, can be used favorably.

Detailed Description Text - DETX (50):
A liquid nutrient culture medium, consisting of 0.1 w/v % peptone, 0.1 w/v % yeasts extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium phosphate, 0.02 w/v % magnesium sulfate, 0.02 w/v % potassium chloride and water, was prepared. About 100 ml aliquots of the nutrient culture medium were placed in 500-ml Erlenmeyer flasks, autoclaved at 120.degree. C. for 20 minutes to effect sterilization, cooled and adjusted to pH 3.0 by the addition of sulphate, and then inoculated with a stock culture of Sulfolobus acidocaldarius ATCC 33909 and incubated at 75.degree. C. for 24 hours under stirring conditions of 130rpm. The resultant cultures were pooled and used as a first seed culture. About 5 liter of a fresh preparation of the same nutrient culture medium as that used in the first seed culture was placed in a 10-liter fermenter, sterilized, cooled to 75.degree. C. and adjusted to pH 3.0, and then inoculated with one v/v % of the first seed culture and incubated at 75.degree. C. for about 48 hours while stirring under aerobic conditions at an aeration of 500 ml/min to obtain a second seed culture. About 250 liter of a fresh preparation of the same nutrient culture medium as that used in the first seed culture was placed in a 300-liter fermenter, sterilized, cooled to 75.degree. C. and adjusted to pH 3.0, and then inoculated with one v/v % of the second seed culture and incubated at 75.degree. C. for about 42 hours while stirring under aerobic conditions at an aeration of 100 ml/min. The present trehalose-releasing enzyme accumulated in the culture were respectively about 0.03 units/ml.

Detailed Description Text - DETX (97):
Preparation of Thermostable Trehalose-releasing Enzyme From Other Microorganisms of the Genus Sulfolobus and its Properties

Detailed Description Text - DETX (99):
According to the method in Experiment 4-3, trehalose was prepared by using these partially purified enzyme preparations, and studied on its structure to find that, similarly as the thermostable trehalose-releasing enzyme from Sulfolobus acidocaldarius (ATCC 33909), every enzyme preparation released trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of 3 or higher.

Detailed Description Text - DETX (102):
A seed culture of Sulfolobus acidocaldarius (ATCC 33909) was incubated by a fermenter for about 42 hours in accordance with the method in Experiment 1. After completion of the incubation, the resultant culture was concentrated with

an SF-membrane to obtain about 5 L of cell suspension. The resultant suspension was treated with "MINI-LAB", a superhigh-pressure cell homogenizer, commercialized by Dainippon Pharmaceutical Co., to disrupt the cells. The resultant solution was centrifuged to recover about 4.8 L of supernatant. To the resultant supernatant was added ammonium sulfate to give a supersaturation degree of about 0.7, and the resultant solution was salted out and centrifuged to obtain a precipitate. The precipitate was dissolved in 10 mM tris-hydrochloride acid buffer (pH 8.5), and dialyzed against a fresh preparation of the same hydrochloride acid buffer. The resultant dialyzed solution was subjected five times to an ion-exchange column chromatography using a column packed with about 2 L of "SEPABEADS FP-DA13" which was equilibrated with said hydrochloride acid buffer, a gel commercialized by Mitsubishi Chemical Industries Ltd., Tokyo, Japan. The objective enzyme adsorbed on the ion exchanger was eluted from the column with a linear gradient buffer supplemented 0M to 0.5M sodium chloride, followed by recovering fractions with enzyme activity which was eluted from the column at about 0.15M sodium chloride. The resultant fractions were concentrated with an SF-membrane, and followed by recovering about 300 ml concentrated enzyme solution containing 32.6 units/ml of thermostable non-reducing saccharide-forming enzyme and 58.5 units/ml of thermostable trehalose-releasing enzyme. The fractions with enzyme activity thus recovered were dialyzed against a fresh preparation of 10 mM Tris-HCl buffer containing 1M ammonium sulfate, and the dialyzed solution thus obtained was centrifuged to remove insoluble substances. The resultant supernatant was subjected five time to hydrophobic column chromatography using a column packed with 350 ml of "BUTYL-TOYOPEARL.RTM. 650", a hydrophobic gel commercialized by Tosoh Corporation, Tokyo, Japan, and followed by separating thermostable non-reducing saccharide-forming enzyme and thermostable trehalose-releasing enzyme. To the suspension of potato starch having a concentration of 15 w/v % was added calcium carbonate to give a final concentration of 0.1 w/w %, adjusted to pH 6.0, admixed with "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, to give a concentration of 0.2 w/w % per g starch and subjected to an enzymatic reaction at 95.degree. C. for 15 min. The resultant mixture was autoclaved for 30 min (2 kg/cm.sup.2), cooled to 58.degree. C., adjusted to pH 5.5, admixed with 2,000 units/g starch of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, 0.5 units/g starch of the above thermostable non-reducing saccharide-forming enzyme and 0.5 units/g starch of the above thermostable trehalose-releasing enzyme, and subjected to an enzymatic reaction for 96 hours. The resultant mixture was kept at 97.degree. C. for 30 min, cooled and filtered. The resultant filtrate was in the usual manner decolored with an activated charcoal, and purified by desalting it with ion-exchange resins in H- and OH-form. The resultant solution was concentrated into a syrup with a concentration of about 60 w/v % in a yield of about 93%, d.s.b. The product contains 71.2% trehalose, 3.0% glucosyltrehalose, 1.3% maltosyltrehalose, 2.9% glucose, 11.1% maltose, 8.5% maltotriose, 2.0% maltooligosaccharides including higher molecular than maltotetraose and inclusive, d.s.b. The product has a mild and high-quality sweetness, as well as an adequate viscosity and moisture-retaining ability, and these properties render it arbitrarily useful in food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer and filler.

Detailed Description Text - DETX (110):

In accordance with the method in Example A-1, a seed culture of a mutant of Sulfolobus acidocaldarius (ATCC 33909) was incubated by a fermenter for about 42 hours. After completion of the incubation, the resultant cells were membrane filtered with an SF-membrane to recover an about 5 L filtrate which was treated with "MINI-LAB", a superhigh-pressure cell homogenizer,

commercialized by Dainippon Pharmaceutical Co., to disrupt the cells. The resultant solution was centrifuged to recover about 4.8 L supernatant. To the resultant supernatant was added ammonium sulfate to give a supersaturation degree of about 0.7, and the resultant solution was salted out and centrifuged to obtain a precipitate. The precipitate was dissolved in 10 mM phosphate buffer (pH 6.5), and dialyzed against a fresh preparation of the same phosphate buffer to recover about 600 ml enzyme solution containing about 15 units/ml of thermostable non-reducing saccharide-forming enzyme and about 12 units/ml of thermostable trehalose-releasing enzyme, and followed by subjecting to a hydrophobic column chromatography to recover 5,850 units of thermostable non-reducing saccharide-forming enzyme and 3,960 units of thermostable trehalose-releasing enzyme. One part by weight of potato starch was admixed with 6 parts by weight of water and 0.01 part by weight of "NEO-SPITASE", .alpha.-amylase, commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan. The resultant mixture was stirred and adjusted to pH 6.2, which was gelatinized and liquidized at a temperature of 85 to 90.degree. C. The resultant liquidized solution was heated at 120.degree. C. for 10 min to inactivate the remaining .alpha.-amylase, cooled to 60.degree. C., adjusted to pH 5.5, admixed with 500 units/g starch of "PROMOZINE", pullulanase commercialized by Novo Nordisk Bioindustry, Copenhagen, Denmark, one unit/g starch of the above thermostable non-reducing saccharide-forming enzyme and one unit/g starch of the above thermostable trehalose-releasing enzyme, and subjected to an enzymatic reaction for 72 hours. The resultant mixture was heated at 97.degree. C. for 30 min to inactivate the remaining enzymes, adjusted to 50.degree. C. and pH 5.0, admixed with 10 units/g starch of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals, Ltd., subjected to an enzymatic reaction for 24 hours, and heated to inactivate the enzyme. The resultant solution was, in a usual manner, decolored, desalted with ion-exchange resins and concentrated into a syrup with a concentration of about 60%. The saccharide solution thus obtained contained 79.5% trehalose, d.s.b. The saccharide solution was column chromatographed in accordance with the method in Example A-2 except that "CG 6000 (Na.sup.+ -form)", a strongly-acidic cation exchange resin commercialized by Japan Organo Co., Ltd., Tokyo, Japan, was used as a resin for fractionation, followed by recovering a trehalose-rich fraction. The fraction contained about 95% trehalose, d.s.b., and it was concentrated into an about 75% solution which was then placed in a crystallizer, admixed with about 2% hydrous crystallized trehalose as a seed crystal and gradually crystallized under stirring conditions. The resultant was placed in a plain plastic-vessel and allowed to stand at an ambient temperature for 3 days to form a block. The resultant block was then pulverized by a cutter to obtain a powdery hydrous crystalline trehalose in a yield of about 70% against the material starch, d.s.b. The product is substantially non-hygroscopic and handles easily, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, excipient, diluent and filler.

Detailed Description Text - DETX (112):

In accordance with the method in Experiment 1, a seed culture of Sulfolobus solfatarius (ATCC 35091) was incubated by a fermenter for about 42 hours. After completion of the incubation, in accordance with the method in Example A-1, the resultant cells were subjected to an SF-membrane filtration and a cell disruption. The resultant supernatant was salted out with ammonium sulfate to obtain a precipitate. The precipitate was dialyzed and followed by subjecting to an ion-exchange column chromatography to recover fractions with enzyme activity. The fractions were concentrated with an UF-membrane and followed by recovering about 150 ml concentrated enzyme solution containing 26.4 units/ml of thermostable non-reducing saccharide-forming enzyme and 57.5 units/ml of

thermostable trehalose-releasing enzyme. The enzyme solution was subjected to a hydrophobic column chromatography to recover 2,650 units of thermostable non-reducing saccharide-forming enzyme and 5,950 units of thermostable trehalose-releasing enzyme. The suspension of potato starch having a concentration of 6% was gelatinized by heating, adjusted to pH 4.5 and 50.degree. C., admixed with 500 units/g starch of isoamylase, and subjected to an enzymatic reaction for 20 hours. The resultant mixture was adjusted to pH 6.5, autoclaved at 120.degree. C. for 10 min, cooled to 95.degree. C., admixed with 0.1 w/w % per g starch of "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, and subjected to an enzymatic reaction for 15 min. The reaction mixture was autoclaved at 130.degree. C. for 30 min, cooled to 65.degree. C., admixed with one unit/g starch of the above non-reducing saccharide-forming enzyme and one unit/g starch of the above trehalose-releasing enzyme, and subjected to an enzymatic reaction for 72 hours. The resultant mixture was kept at 97.degree. C. for 30 min, adjusted to pH 5.0 and 50.degree. C., admixed with 10 units/g starch of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals, Ltd., subjected to an enzymatic reaction for 24 hours, and heated to inactivate the enzyme. The resultant solution was, in a usual manner, decolored, desalted with ion-exchange resins and concentrated into a syrup with a concentration of about 60%. The saccharide solution thus obtained contained 80.9% trehalose, d.s.b. The saccharide solution was concentrated to give a concentration of about 84%, and then placed in a crystallizer, admixed with about 2% hydrous crystalize trehalose as a seed crystal and gradually crystallized under stirring conditions. The resultant was placed in a plain plastic-vessel and allowed to stand at an ambient temperature for 3 days to form a block. The resultant block was then pulverized by a cutter to obtain a powdery hydrous crystalline trehalose in a yield of about 90% against the material starch, d.s.b. The product is substantially non-hygroscopic and handles easily, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, excipient, diluent and filler.

Claims Text - CLTX (1):

1. A biologically pure culture of a microorganism capable of producing a thermostable trehalose-releasing enzyme which specifically hydrolyzes the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3, but which does not form trehalose from starch wherein said microorganism is selected from the group consisting of microorganisms of the genus Sulfolobus and mutants thereof, with the exclusion of Sulfolobus acidocaldarius strains having ATCC numbers 33909 and 49426, and Sulfolobus solfataricus strains having ATCC numbers 35091 and 35092, wherein said biologically pure culture is obtained by culturing said microorganism in a nutrient culture medium to produce said enzyme and removing the impurities from the culture to increase the purity of said enzyme, said biologically pure culture having at least 0.03 unit/ml of enzyme activity.

US-PAT-NO: 6136571

DOCUMENT-IDENTIFIER: US 6136571 A

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 24, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 499531

DATE FILED: February 10, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/198,672 filed on Nov. 23, 1998, which is a continuation-in-part of U.S. Ser. No. 09/107,657 filed on Jun. 30, 1998, now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/979,673 filed on Nov. 26, 1997, now abandoned, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 435/96, 127/40 , 127/55 , 435/98 , 435/99

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

15 Claims, 5 Drawing figures

Exemplary Claim Number: 1,8

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (50):

Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae Sulfolobus solfataricus KM1.

US-PAT-NO: 6129788

DOCUMENT-IDENTIFIER: US 6129788 A

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 10, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 198672

DATE FILED: November 23, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/107,657 filed on Jun. 30, 1998, now abandoned, which is a continuation of Ser. No. 08/979,673 filed on Nov. 26, 1997, now abandoned, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 127/40, 435/105 , 435/96 , 435/98

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and recirculation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

16 Claims, 5 Drawing figures

Exemplary Claim Number: 1,9

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (50):

Another enzymatic process for producing trehalose from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using trehalose-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic

archae Sulfolobus solfataricus KM1.

US-PAT-NO: 6100073

DOCUMENT-IDENTIFIER: US 6100073 A

TITLE: Acid-stable and thermo-stable enzymes derived from sulfolobus species

DATE-ISSUED: August 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Deweer; Philippe	Aalst	N/A	N/A	BE
Amory; Antione	Rixensart	N/A	N/A	BE

APPL-NO: 08/ 765939

DATE FILED: September 29, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9414224	July 14, 1994

PCT-DATA:
APPL-NO: PCT/EP95/02703
DATE-FILED: July 7, 1995
PUB-NO: WO96/02633
PUB-DATE: Feb 1, 1996
371-DATE: Sep 29, 1997
102(E)-DATE:Sep 29, 1997

US-CL-CURRENT: 435/99, 435/105 , 435/202 , 435/203 , 435/204 , 435/96

ABSTRACT:

Novel acid-stable and thermo-stable enzymes having .alpha.-1,4 hydrolytic activity and a .alpha.-1,6 hydrolytic activity which are derived from strains of the genus Sulfolobus. These enzymes are capable of expressing high levels of .alpha.-1,4 hydrolytic activity, including the maximum .alpha.-1,4 hydrolytic activity thereof, at highly acidic pHs of between about 2.5 and about 4.5. These .alpha.-amylases are further capable of expressing high levels of .alpha.-1,4 hydrolytic activity, including the maximum .alpha.-1,4 hydrolytic activity thereof, at high temperatures of between about 90.degree. C. and about 120.degree. C. Particularly disclosed herein are such enzymes which are derived from strains of the species S. acidocaldarius and, in particular, Sulfolobus acidocaldarius DSM 639. Modified starch degradation (liquefaction and saccharification) processes using these novel enzymes are also disclosed herein.

33 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (18):

Further problems presented by the use of the .alpha.-amylase derived from S. solfataricus in liquefaction is that it is produced intracellularly and that it catalyzes the synthesis of trehalose. In fact, we are not aware of any acid-stable .alpha.-amylases which are extracellularly-secreted by any species of Sulfolobus.

US-PAT-NO: 6027918

DOCUMENT-IDENTIFIER: US 6027918 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitsuzumi; Hitoshi	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP

APPL-NO: 08/ 798269

DATE FILED: February 11, 1997

PARENT-CASE:

This is a division of co-pending parent application Ser. No. 08/505,377 filed Jul. 21, 1995.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190180	September 21, 1994
JP	7-109128	April 11, 1995
JP	7-189760	July 4, 1995

US-CL-CURRENT: 435/69.2, 435/183 , 435/200 , 435/252.33 , 435/320.1 , 435/69.1 , 435/71.1 , 435/71.2 , 536/23.1 , 536/23.2 , 536/23.7

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 54,000-64,000 daltons and a pI of about 5.6-6.6, and releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. The enzyme has a satisfactorily-high thermostability, i.e. it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of trehalose on an industrial scale and in a satisfactorily-high yield.

13 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (9):

In view of the foregoing, the present inventors screened thermostable enzyme with a satisfactory activity and have found that enzymes produced from

microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently release trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. These microorganisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce trehalose from those non-reducing saccharides.

Detailed Description Text - DETX (8):

The present invention has been made based on the finding of a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

US-PAT-NO: 5976856

DOCUMENT-IDENTIFIER: US 5976856 A

TITLE: Recombinant thermostable enzyme which forms non-reducing saccharide from reducing amylaceous saccharide

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maruta; Kazuhiko	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP

APPL-NO: 08/ 505448

DATE FILED: July 21, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190183	July 21, 1994
JP	7-189706	July 4, 1995

US-CL-CURRENT: 435/201, 435/101 , 435/193 , 435/200 , 435/205 , 435/96 , 435/97 , 435/99

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

1 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (9):

In view of the foregoing, the present inventors screened thermostable enzyme with such a novel enzyme activity and have found that enzymes produced from microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently produce such non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides. These micro-organisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large

scale culture to industrially produce non-reducing saccharides having a trehalose structure as an end unit.

Detailed Description Text - DETX (8):

The present invention has been accomplished based on the finding of a novel enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

US-PAT-NO: 5922578

DOCUMENT-IDENTIFIER: US 5922578 A

TITLE: Recombinant thermostable enzyme which forms non-reducing saccharide from reducing amylaceous saccharide

DATE-ISSUED: July 13, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maruta; Kazuhiko	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP

APPL-NO: 08/ 840236

DATE FILED: April 11, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a division of parent application Ser. No. 08/505,448, filed Jul. 21, 1995.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190183	July 21, 1994
JP	7-189706	July 4, 1995

US-CL-CURRENT: 435/97, 435/101 , 435/200 , 435/201 , 435/202 , 435/205 , 435/96 , 435/99

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pl of about 5.4-6.4, and forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

7 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (9):
In view of the foregoing, the present inventors screened thermostable enzyme

with such a novel enzyme activity and have found that enzymes produced from microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently produce such non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides. These micro-organisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce non-reducing saccharides having a trehalose structure as an end unit.

Detailed Description Text - DETX (8):

The present invention has been accomplished based on the finding of a novel enzyme which forms non-reducing saccharides having a trehalose structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

US-PAT-NO: 5919668

DOCUMENT-IDENTIFIER: US 5919668 A

TITLE: Non-reducing saccharide and its production and use

DATE-ISSUED: July 6, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mandai; Takahiko	Okayama	N/A	N/A	JP
Shibuya; Takashi	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP
Miyake; Toshio	Okayama	N/A	N/A	JP

APPL-NO: 08/ 495030

DATE FILED: June 27, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of Ser. No. 08/253,171, filed Jun. 2, 1994, now U.S. Pat. No. 5,472,863.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-165815	June 27, 1994
JP	7-116583	April 19, 1995

US-CL-CURRENT: 435/97, 435/100 , 435/101 , 435/72 , 435/74 , 435/95 , 435/96 , 435/98 , 435/99 , 536/123.1 , 536/123.13

ABSTRACT:

In the production of non-reducing saccharides such as trehalose, alpha-glycosyl trehaloses and alpha-glycosyl alpha-glycosides where a solution of liquefied starch is subjected either to non- educing saccharide-forming enzyme or non-reducing saccharide-forming enzyme and trehalose-releasing enzyme, combinations with starch-debranching enzyme and/or cyclomaltodextrin glucanotransferase improve the yields for such non-reducing saccharides to levels which are hardly attainable only with reducing-saccharide-forming enzyme and trehalose-releasing enzyme. The non-reducing saccharides and less reducing reducing saccharides containing the same commonly bear a variety of desirable properties which make them useful in a variety of compositions including food products, cosmetics and medicines.

22 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Detailed Description Text - DETX (2):

First, the non-reducing saccharide-forming enzymes feasible in the present invention are those which are capable of forming alpha-glycosyl trehaloses from one or more reducing partial starch hydrolysates with glucose polymerization degrees of 3 or higher in solutions of starch which has been liquefied to a relatively low DE: Examples of such an enzyme are those derived from micro-organisms of the genera Rhizobium, Arthrobacter, Brevibacterium, Flabobacterium, Micrococcus, Curtobacterium, Mycobacterium and Terrabacter which are disclosed in Japan Patent Application No. 349,216/93. If necessary, heat-resistant non-reducing saccharide-forming enzymes, for example, those from the genus Sulfolobus as disclosed in Japanese Patent Application No. 166,011/94 by the same applicant, can be arbitrarily used. The trehalose-releasing enzymes are such as those which specifically hydrolyze the linkages between the trehalose moieties and the other moieties in alpha-glycosyl trehaloses which have been formed by subjecting a solution of liquefied starch to non-reducing saccharide-forming enzyme. Examples of such an enzyme are those derived from the genera Rhizobium, Arthrobacter, Brevibacterium and Micrococcus which are all disclosed in Japanese Patent Application No. 79,291/94. If necessary, heat-resistant trehalose-releasing enzymes, for example, those as disclosed in Japanese Patent Application No. 166,126/94 by the same applicant, can be arbitrarily used. To prepare non-reducing saccharide-forming enzyme and/or trehalose-releasing enzyme, micro-organisms capable of producing either of both of the enzymes are cultivated.

US-PAT-NO: 5863771

DOCUMENT-IDENTIFIER: US 5863771 A

TITLE: Saccharide composition comprising maltooligosylturanose and maltooligosylpalatinose, its preparation and uses

DATE-ISSUED: January 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Aga; Hajime	Okayama	N/A	N/A	JP
Shibuya; Takashi	Okayama	N/A	N/A	JP
Fukuda; Shigeharu	Okayama	N/A	N/A	JP
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APPL-NO: 08/ 810363

DATE FILED: March 3, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	8-070915	March 4, 1996
JP	8-099571	March 29, 1996

US-CL-CURRENT: 435/101, 426/658 , 435/100 , 435/72 , 435/99 , 514/54 , 514/61 , 514/777 , 536/1.11 , 536/123.1 , 536/124 , 536/4.1

ABSTRACT:

A saccharide composition comprising maltooligosyl derivatives of turanose and palatinose which can be readily produced, separated, and purified in a relatively-high yield from maltooligosylsucrose by allowing non-reducing saccharide-forming enzymes to act on aqueous solutions containing maltoolig 58Yosucrose. These saccharides are reducing oligosaccharides with a mild and high-quality sweetness and can be used orally and parenterally, as well as being readily metabolized and used by living bodies.

17 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Detailed Description Text - DETX (2):

As is disclosed in Japanese Patent Laid-Open No. 143,876/95, the non-reducing saccharide-forming enzymes usable in the present invention are produced from Rhizobium sp. M-11 (FERM BP-4130), Arthrobacter sp. Q36 (FERM BP-4316), Brevibacterium helvolum (ATCC 11822), Flavobacterium aquatile (IFO 3772), Micrococcus roseus (ATCC 186), Curtobacterium citreum (IFO 15231), Mycobacterium smegmatis (ATCC 19420), Terrabacter tumescens (IFO 12960), and other microorganisms of the genus Sulfolobus as disclosed in Japanese Patent Application No. 166,011/94. These enzymes are intramolecular saccharide-transferring enzymes which convert or rearrange

maltooligosaccharides into maltooligosyltrehalose. The equilibrium point of these enzymes predominantly inclines to the side of forming maltooligosyltrehalose: For example, they produce maltotriosyltrehalose from maltopentaose as a substrate in a yield of at least about 90 w/w %, on a dry solid basis (the wording "w/w %, on a dry solid basis" as referred to in the present invention will be abbreviated as "%", unless specified otherwise).

Detailed Description Text - DETX (10):

The method for assaying the activity of the non-reducing saccharide-forming enzymes used in the present invention is as follows: Add one ml of an enzyme solution to 4 ml of 1.25 w/v % maltopentaose as a substrate dissolved in 50 mM phosphate buffer (pH 7.0), react the mixture at 40.degree. C. for 60 min, heat the reaction mixture at 100.degree. C. for 10 min to suspend the enzymatic reaction, precisely dilute the mixture with deionized water by 10-fold, and assay the reducing power of the dilution by the Somogyi-Nelson's method. As a control, an enzyme solution which had been heated at 100.degree. C. for 10 min to inactivate the enzyme is assayed similarly as above. One unit activity of the enzyme is defined as the amount of enzyme which eliminates the reducing power of that of one micromole of maltopentaose per minute when assayed by the above method. In the case of non-reducing saccharide-forming enzymes from microorganisms of the genus Sulfolobus, the enzymes are reacted at 60.degree. C. and pH 5.5, then inactivated by heating at 100.degree. C. for 30 min.

Detailed Description Text - DETX (51):

In accordance with the methods in Experiments 2 and 3, purified non-reducing saccharide-forming enzymes from Arthrobacter sp. Q36 (FERM BP-4316) and Rhizobium sp. M-11 (FERM BP-4130) were prepared, and a partially purified non-reducing saccharide-forming enzyme from Sulfolobus acidocaldarius (ATCC 33909) was obtained by column chromatography using "DEAE-TOYOPEARL.RTM. 650". These enzymes were allowed to act on a 20% maltotetraosylsucrose solution for 96 hours under the conditions as shown in Table 2. The results were in Table 2.

Detailed Description Text - DETX (80):

Two and half parts by weight of sucrose and 2.5 parts by weight of maltopentaose commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, were dissolved by heating in 5 parts by weight of water, and the solution was adjusted to pH 6.5 and 45.degree. C., admixed with one unit/g sucrose of levansucrase from a strain of the genus Bacillus, enzymatically reacted for 20 hours, and heated to inactivate the remaining enzyme. The reaction mixture was in a conventional manner decolored with an activated charcoal, desalted and purified with ion exchangers in H--and OH--form, and fractionated using an "ODS" column for fractionation to obtain a high-purity maltotetraosyl-sucrose solution. To the solution was added 50 units/g dry matter of a thermostable non-reducing saccharide-forming enzyme from a strain of the genus Sulfolobus obtained by the method in Example for Reference 3, enzymatically reacted at 65.degree. C. for 20 hours, and heated to inactivate the remaining enzyme. The reaction mixture was in a usual manner decolored with an activated charcoal, desalted and purified with ion exchangers in H--and OH--form, concentrated, and spray dried to obtain a powder containing maltooligosylturanose and maltooligosylpalatinose in a yield of about 85%. The powder with non-crystallinity contained about 55% maltotetraosylturanose and maltotetraosylpalatinose and about 18% maltotetraosylsucrose. Because the powder has a satisfactory sweetness, it can be arbitrarily used in food products, cosmetics, and pharmaceuticals.

US-PAT-NO: 5856146

DOCUMENT-IDENTIFIER: US 5856146 A

TITLE: Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide

DATE-ISSUED: January 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitsuzumi; Hitoshi	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP

APPL-NO: 08/ 505377

DATE FILED: July 21, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-190180	July 21, 1994
JP	7-109128	April 11, 1995
JP	7-189760	July 4, 1995

US-CL-CURRENT: 435/97, 435/100 , 435/195 , 435/200 , 435/201 , 435/253.3 , 435/822 , 530/350 , 530/825

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 54,000-64,000 daltons and a pl of about 5.6-6.6, and releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. The enzyme has a satisfactorily-high thermostability, i.e. it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of trehalose on an industial scale and in a satisfactorily-high yield.

6 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

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Brief Summary Text - BSTX (9):

In view of the foregoing, the present inventors screened thermostable enzymes with a satisfactory activity and have found that enzymes produced from microorganisms of the genus Sulfolobus including Sulfolobus acidocaldarius (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently release trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. These microorganisms, however, are not sufficient in enzyme productivity, and this requires a relatively-large

scale culture to industrially produce trehalose from those non-reducing saccharides.

Detailed Description Text - DETX (8):

The present invention has been made based on the finding of a novel enzyme which releases trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species Sulfolobus acidocaldarius (ATCC 33909). The present inventors isolated such an enzyme by using in combination various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the enzyme is a polypeptide with the following physicochemical properties:

US-PAT-NO: 5789392

DOCUMENT-IDENTIFIER: US 5789392 A

TITLE: Saccharide composition with reduced reducibility, and preparation and uses thereof

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shibuya; Takashi	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP
Miyake; Toshio	Okayama	N/A	N/A	JP

APPL-NO: 08/ 883079

DATE FILED: June 26, 1997

PARENT-CASE:

This is a divisional of parent application Ser. No. 08/492,961, filed Jun. 20, 1995, now abandoned U.S. Pat. No. 5,681,826.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-180393	June 27, 1994

US-CL-CURRENT: 514/54, 536/123.1 , 536/124

ABSTRACT:

A saccharide composition with a reduced reducibility which is prepared by hydrogenating a saccharide mixture comprising reducing saccharides and non-reducing saccharides consisting of trehalose and/or saccharides having a trehalose structure. The saccharide composition has a satisfactory sweetness, taste and stability, and is substantially free from reducibility, so that it can be freely used in a variety of compositions such as foods, cosmetics and pharmaceuticals which are susceptible to reduction.

9 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Detailed Description Text - DETX (2):

The non-reducing saccharide-forming enzymes usable in the present invention include those which can form .alpha.-glycosyltrehalose from one or more reducing amylaceous partial starch hydrolysates selected from those with a glucose polymerization degree of 3 or more which are contained in liquefied starch solutions with a relatively-low DE. Examples of such enzymes are those derived from microorganisms of the genera Rhizobium, Arthrobacter, Brevibacterium, Flavobacterium, Micrococcus, Curtobacterium, Mycobacterium and

Terrabacter as disclosed in Japanese Patent Application No.349,216/93. If necessary, thermostable non-reducing saccharide-forming enzymes can be arbitrarily used in the present invention. For example, a thermostable non-reducing saccharide-forming enzyme derived from a microorganism of the genus Sulfolobus as disclosed in Japanese Patent Application No.166,011/94, titled "Thermostable non-reducing saccharide-forming enzyme, and its preparation and uses", applied for by the present applicant on the day of Jun. 24, 1994, can be arbitrarily used. Any enzyme, which specifically hydrolyzes the linkage between a trehalose moiety and others in .alpha.-glycosyltrehalose formed by allowing a non-reducing saccharide-forming enzyme to act on a liquefied starch solution, can be used as a trehalose-releasing enzyme in the present invention: For example, those derived from microorganisms of the genera Rhizobium, Arthrobacter, Brevibacterium and Micrococcus as disclosed in Japanese Patent Application No.79,291/94 can be arbitrarily used. If necessary, thermostable trehalose-releasing enzymes such as that derived from a microorganism of the genus Sulfolobus as disclosed in Japanese Patent Application No.166,126/94, applied for by the present applicant on the day of Jun. 25, 1994, can be arbitrarily used in the present invention.

US-PAT-NO: 5723327

DOCUMENT-IDENTIFIER: US 5723327 A

TITLE: Thermostable trehalose-releasing enzyme, and its preparation and uses

DATE-ISSUED: March 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ikegami; Shouji	Okayama	N/A	N/A	JP
Kubota; Michio	Okayama	N/A	N/A	JP
Sugimoto; Toshiyuki	Okayama	N/A	N/A	JP
Miyake; Toshio	Okayama	N/A	N/A	JP

APPL-NO: 08/ 485132

DATE FILED: June 7, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-166126	June 25, 1994
JP	7-109130	April 11, 1995

US-CL-CURRENT: 435/201, 435/100 , 435/193 , 435/195 , 435/200 , 536/123.13

ABSTRACT:

Disclosed are novel thermostable trehalose-releasing enzyme, and its preparations and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of hydrolyzing at a temperature of over 55.degree. C. the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Trehalose and compositions containing the same are extensively useful in food products, cosmetics and pharmaceuticals.

15 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Abstract Text - ABTX (1):

Disclosed are novel thermostable trehalose-releasing enzyme, and its preparations and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of hydrolyzing at a temperature of over 55.degree. C. the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher. Trehalose and compositions containing the same

are extensively useful in food products, cosmetics and pharmaceuticals.

Brief Summary Text - BSTX (9):
On the other hand, since a thermostable enzyme can maintain its activity at a relatively-high temperature, contamination during the enzymatic reaction is less of a concern and the retrogradation of partial starch hydrolysates is scarcely caused. As a source of thermostable enzymes, thermophilic microorganisms can be generally considered. Regarding a preparation of trehalose using thermophilic microorganisms, as described in Biotechnology Letters, Vol. 12, pp. 431-432 (1990) and Biotech Forum Europe, Vol. 8, pp. 201-203 (1991), it was reported that the partially purified enzyme preparation obtainable from the cell and cell extract of Sulfolobus solfataricus (ATCC 49155) forms glucose and trehalose when allowed to act on substrate such as amylose and soluble starch. A purification of such an enzyme preparation can not be completed, however, as the physicochemical properties of the enzyme thus prepared are not sufficiently indicated and the action of the enzyme has not been clarified, and only a preparation of trehalose is indicated. Thus, there has been a great demand to establish a novel preparation of trehalose by utilizing a thermostable enzyme capable of acting at a temperature of over 55.degree. C.

Brief Summary Text - BSTX (13):
As a result, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, these as disclosed in Japanese Patent Application No. 166,011/94, produce a thermostable non-reducing saccharide-forming enzyme and also a novel thermostable trehalose-releasing enzyme which are capable of acting at a temperature of over 55.degree. C., and found that the objective preparation of trehalose at a temperature of over 55.degree. C. is readily conducted by allowing the thermostable non-reducing saccharide enzyme together with this novel thermostable trehalose-releasing enzyme to act on reducing partial starch hydrolysates. The present inventors also found that trehalose is readily preparable by allowing the thermostable non-reducing saccharide-forming enzyme together with the novel thermostable trehalose-releasing enzyme to act on reducing partial starch hydrolysates and subjecting to the action of glucoamylase or .alpha.-glucosidase to obtain reacted solutions containing trehalose with a relatively-high purity. Thus, the present inventors accomplished this invention.

Detailed Description Text - DETX (4):
Now, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, are capable of producing a novel thermostable trehalose-releasing enzyme.

Detailed Description Text - DETX (5):
In addition to the above-mentioned microorganisms, other strains of the genus Sulfolobus and their mutants can be arbitrarily used in the present invention as long as they produce a thermostable trehalose-releasing enzyme which specifically hydrolyses the linkage between a trehalose moiety and the remaining glycosyl moiety in a non-reducing saccharide having a trehalose structure as an end unit and having a degree of glucose polymerization of 3 or higher.

Detailed Description Text - DETX (27):
As non-reducing saccharide-forming enzymes which forms non-reducing saccharides having a trehalose structure and having a degree of glucose polymerization of 3 or higher when allowed to act on reducing partial starch hydrolysates, those derived from Rhizobium sp. M-11 or Arthrobactor sp. Q36 as disclosed in Japanese Patent Application No. 349,216/93, can be used, however, in case that an enzymatic reaction proceeds at a temperature of over 55.degree. C., the thermostable non-reducing saccharide-forming enzyme which belongs to the group of the genus Sulfolobus, disclosed in Japanese Patent Application No. 166,011/94, can be used favorably.

Detailed Description Text - DETX (50):
A liquid nutrient culture medium, consisting of 0.1 w/v % peptone, 0.1 w/v % yeasts extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium phosphate, 0.02 w/v % magnesium sulfate, 0.02 w/v % potassium chloride and water, was prepared. About 100 ml aliquots of the nutrient culture medium were placed in 500-ml Erlenmeyer flasks, autoclaved at 120.degree. C. for 20 minutes to effect sterilization, cooled and adjusted to pH 3.0 by the addition of sulphate, and then inoculated with a stock culture of Sulfolobus acidocaldarius ATCC 33909 and incubated at 75.degree. C. for 24 hours under stirring conditions of 130 rpm. The resultant cultures were pooled and used as a first seed culture. About 5 liter of a fresh preparation of the same nutrient culture medium as that used in the first seed culture was placed in a 10-liter fermenter, sterilized, cooled to 75.degree. C. and adjusted to pH 3.0, and then inoculated with one v/v % of the first seed culture and incubated at 75.degree. C. for about 48 hours while stirring under aerobic conditions at an aeration of 500 ml/min to obtain a second seed culture. About 250 liter of a fresh preparation of the same nutrient culture medium as that used in the first seed culture was placed in a 300-liter fermenter, sterilized, cooled to 75.degree. C. and adjusted to pH 3.0, and then inoculated with one v/v % of the second seed culture and incubated at 75.degree. C. for about 42 hours while stirring under aerobic conditions at an aeration of 100 ml/min. The present trehalose-releasing enzyme accumulated in the culture were respectively about 0.03 units/ml.

Detailed Description Text - DETX (97):
Preparation of thermostable trehalose-releasing enzyme from other microorganisms of the genus Sulfolobus and its properties

Detailed Description Text - DETX (99):
According to the method in Experiment 4-3, trehalose was prepared by using these partially purified enzyme preparations, and studied on its structure to find that, similarly as the thermostable trehalose-releasing enzyme from Sulfolobus acidocaldarius (ATCC 33909), every enzyme preparation released trehalose from non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of 3 or higher.

Detailed Description Text - DETX (102):
A seed culture of Sulfolobus acidocaldarius (ATCC 33909) was incubated by a fermenter for about 42 hours in accordance with the method in Experiment 1. After completion of the incubation, the resultant culture was concentrated with an SF-membrane to obtain about 5 L of cell suspension. The resultant suspension was treated with "MINI-LAB", a superhigh-pressure cell homogenizer, commercialized by Dainippon Pharmaceutical Co., to disrupt the cells. The resultant solution was centrifuged to recover about 4.8 L of supernatant. To

the resultant supernatant was added ammonium sulfate to give a supersaturation degree of about 0.7, and the resultant solution was salted out and centrifuged to obtain a precipitate. The precipitate was dissolved in 10 mM tris-hydrochloride acid buffer (pH 8.5), and dialyzed against a fresh preparation of the same hydrochloride acid buffer. The resultant dialyzed solution was subjected five times to an ion-exchange column chromatography using a column packed with about 2 L of "SEPABEADS FP-DA13" which was equilibrated with said hydrochloride acid buffer, a gel commercialized by Mitsubishi Chemical Industries Ltd., Tokyo, Japan. The objective enzyme adsorbed on the ion exchanger was eluted from the column with a linear gradient buffer supplemented 0M to 0.5M sodium chloride, followed by recovering fractions with enzyme activity which was eluted from the column at about 0.15M sodium chloride. The resultant fractions were concentrated with an SF-membrane, and followed by recovering about 300 ml concentrated enzyme solution containing 32.6 units/ml of thermostable non-reducing saccharide-forming enzyme and 58.5 units/ml of thermostable trehalose-releasing enzyme. The fractions with enzyme activity thus recovered were dialyzed against a fresh preparation of 10 mM Tris-HCl buffer containing 1M ammonium sulfate, and the dialyzed solution thus obtained was centrifuged to remove insoluble substances. The resultant supernatant was subjected five time to hydrophobic column chromatography using a column packed with 350 ml of "BUTYL-TOYOPEARL.RTM. 650", a hydrophobic gel commercialized by Tosoh Corporation, Tokyo, Japan, and followed by separating thermostable non-reducing saccharide-forming enzyme and thermostable trehalose-releasing enzyme. To the suspension of potato starch having a concentration of 15 w/v % was added calcium carbonate to give a final concentration of 0.1 w/w %, adjusted to pH 6.0, admixed with "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, to give a concentration of 0.2 w/w % per g starch and subjected to an enzymatic reaction at 95.degree. C. for 15 min. The resultant mixture was autoclaved for 30 min (2 kg/cm.sup.2), cooled to 58.degree. C., adjusted to pH 5.5, admixed with 2,000 units/g starch of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, 0.5 units/g starch of the above thermostable non-reducing saccharide-forming enzyme and 0.5 units/g starch of the above thermostable trehalose-releasing enzyme, and subjected to an enzymatic reaction for 96 hours. The resultant mixture was kept at 97.degree. C. for 30 min, cooled and filtered. The resultant filtrate was in the usual manner decolored with an activated charcoal, and purified by desalting it with ion-exchange resins in H- and OH-form. The resultant solution was concentrated into a syrup with a concentration of about 60 w/v % in a yield of about 93%, d.s.b. The product contains 71.2% trehalose, 3.0% glucosyltrehalose, 1.3% maltosyltrehalose, 2.9% glucose, 11.1% maltose, 8.5% maltotriose, 2.0% maltooligosaccharides including higher molecular than maltotetraose and inclusive, d.s.b. The product has a mild and high-quality sweetness, as well as an adequate viscosity and moisture-retaining ability, and these properties render it arbitrarily useful in food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer and filler.

Detailed Description Text - DETX (110):

In accordance with the method in Example A-1, a seed culture of a mutant of Sulfolobus acidocaldarius (ATCC 33909) was incubated by a fermenter for about 42 hours. After completion of the incubation, the resultant cells were membrane filtered with an SF-membrane to recover an about 5 L filtrate which was treated with "MINI-LAB", a superhigh-pressure cell homogenizer, commercialized by Dainippon Pharmaceutical Co., to disrupt the cells. The resultant solution was centrifuged to recover about 4.8 L supernatant. To the resultant supernatant was added ammonium sulfate to give a supersaturation degree of about 0.7, and the resultant solution was salted out and centrifuged

to obtain a precipitate. The precipitate was dissolved in 10 mM phosphate buffer (pH 6.5), and dialyzed against a fresh preparation of the same phosphate buffer to recover about 600 ml enzyme solution containing about 15 units/ml of thermostable non-reducing saccharide-forming enzyme and about 12 units/ml of thermostable trehalose-releasing enzyme, and followed by subjecting to a hydrophobic column chromatography to recover 5,850 units of thermostable non-reducing saccharide-forming enzyme and 3,960 units of thermostable trehalose-releasing enzyme. One part by weight of potato starch was admixed with 6 parts by weight of water and 0.01 part by weight of "NEO-SPITASE", .alpha.-amylase, commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan. The resultant mixture was stirred and adjusted to pH 6.2, which was gelatinized and liquidized at a temperature of 85.degree. to 90.degree. C. The resultant liquidized solution was heated at 120.degree. C. for 10 min to inactivate the remaining .alpha.-amylase, cooled to 60.degree. C., adjusted to pH 5.5, admixed with 500 units/g starch of "PROMOZINE", pullulanase commercialized by Novo Nordisk Bioindustry, Copenhagen, Denmark, one unit/g starch of the above thermostable non-reducing saccharide-forming enzyme and one unit/g starch of the above thermostable trehalose-releasing enzyme, and subjected to an enzymatic reaction for 72 hours. The resultant mixture was heated at 97.degree. C. for 30 min to inactivate the remaining enzymes, adjusted to 50.degree. C. and pH 5.0, admixed with 10 units/g starch of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals, Ltd., subjected to an enzymatic reaction for 24 hours, and heated to inactivate the enzyme. The resultant solution was, in a usual manner, decolored, desalted with ion-exchange resins and concentrated into a syrup with a concentration of about 60%. The saccharide solution thus obtained contained 79.5% trehalose, d.s.b. The saccharide solution was column chromatographed in accordance with the method in Example A-2 except that "CG 6000 (Na.sup.+ -form)", a strongly-acidic cation exchange resin commercialized by Japan Organo Co., Ltd., Tokyo, Japan, was used as a resin for fractionation, followed by recovering a trehalose-rich fraction. The fraction contained about 95% trehalose, d.s.b., and it was concentrated into an about 75% solution which was then placed in a crystallizer, admixed with about 2% hydrous crystallized trehalose as a seed crystal and gradually crystallized under stirring conditions. The resultant was placed in a plain plastic-vessel and allowed to stand at an ambient temperature for 3 days to form a block. The resultant block was then pulverized by a cutter to obtain a powdery hydrous crystalline trehalose in a yield of about 70% against the material starch, d.s.b. The product is substantially non-hygroscopic and handles easily, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, excipient, diluent and filler.

Detailed Description Text - DETX (112):

In accordance with the method in Experiment 1, a seed culture of Sulfolobus solfatarius (ATCC 35091) was incubated by a fermenter for about 42 hours. After completion of the incubation, in accordance with the method in Example A-1, the resultant cells were subjected to an SF-membrane filtration and a cell disruption. The resultant supernatant was salted out with ammonium sulfate to obtain a precipitate. The precipitate was dialyzed and followed by subjecting to an ion-exchange column chromatography to recover fractions with enzyme activity. The fractions were concentrated with an UF-membrane and followed by recovering about 150 ml concentrated enzyme solution containing 26.4 units/ml of thermostable non-reducing saccharide-forming enzyme and 57.5 units/ml of thermostable trehalose-releasing enzyme. The enzyme solution was subjected to a hydrophobic column chromatography to recover 2,650 units of thermostable non-reducing saccharide-forming enzyme and 5,950 units of thermostable trehalose-releasing enzyme. The suspension of potato starch having a

concentration of 6% was gelatinized by heating, adjusted to pH 4.5 and 50.degree. C., admixed with 500 units/g starch of isoamylase, and subjected to an enzymatic reaction for 20 hours. The resultant mixture was adjusted to pH 6.5, autoclaved at 120.degree. C. for 10 min, cooled to 95.degree. C., admixed with 0.1 w/w % per g starch of "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, and subjected to an enzymatic reaction for 15 min. The reaction mixture was autoclaved at 130.degree. C. for 30 min, cooled to 65.degree. C., admixed with one unit/g starch of the above non-reducing saccharide-forming enzyme and one unit/g starch of the above trehalose-releasing enzyme, and subjected to an enzymatic reaction for 72 hours. The resultant mixture was kept at 97.degree. C. for 30 min, adjusted to pH 5.0 and 50.degree. C., admixed with 10 units/g starch of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals, Ltd., subjected to an enzymatic reaction for 24 hours, and heated to inactivate the enzyme. The resultant solution was, in a usual manner, decolored, desalted with ion-exchange resins and concentrated into a syrup with a concentration of about 60%. The saccharide solution thus obtained contained 80.9% trehalose, d.s.b. The saccharide solution was concentrated to give a concentration of about 84%, and then placed in a crystallizer, admixed with about 2% hydrous crystalize trehalose as a seed crystal and gradually crystallized under stirring conditions. The resultant was placed in a plain plastic-vessel and allowed to stand at an ambient temperature for 3 days to form a block. The resultant block was then pulverized by a cutter to obtain a powdery hydrous crystalline trehalose in a yield of about 90% against the material starch, d.s.b. The product is substantially non-hygroscopic and handles easily, and these render it arbitrarily useful in a variety of compositions such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, excipient, diluent and filler.

US-PAT-NO: 5714368

DOCUMENT-IDENTIFIER: US 5714368 A

TITLE: Thermostable non-reducing saccharide-forming enzyme its production and uses

DATE-ISSUED: February 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 08/ 466434

DATE FILED: June 6, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-166011	June 24, 1994

US-CL-CURRENT: 435/201, 435/100 , 435/101 , 435/200 , 514/53 , 514/54 , 514/60 , 536/123.13 , 536/124

ABSTRACT:

Disclosed are novel thermostable non-reducing saccharides-forming enzyme, its preparation and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of forming non-reducing saccharides having a trehalose structure as an end unit when allowed to act on reducing partial starch hydrolysates at a temperature of over 55.degree. C. Glucoamylase and .alpha.-glucosidase readily yield trehalose when allowed to act on the non-reducing saccharides. These non-reducing saccharides and trehalose are extensively useful in food products, cosmetics and pharmaceuticals.

23 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Abstract Text - ABTX (1):
Disclosed are novel thermostable non-reducing saccharides-forming enzyme, its preparation and uses. The enzyme is obtainable from the culture of microorganisms such as Sulfolobus acidocaldarius (ATCC 33909 and ATCC 49426) and Sulfolobus solfataricus (ATCC 35091 and ATCC 35092), and capable of forming non-reducing saccharides having a trehalose structure as an end unit when allowed to act on reducing partial starch hydrolysates at a temperature of over 55.degree. C. Glucoamylase and .alpha.-glucosidase readily yield trehalose

when allowed to act on the non-reducing saccharides. These non-reducing saccharides and trehalose are extensively useful in food products, cosmetics and pharmaceuticals.

Brief Summary Text - BSTX (15):

To attain the aforementioned object, the present inventors have extensively screened microorganisms capable of producing a novel thermostable non-reducing saccharide-forming enzyme, which forms non-reducing saccharides having a trehalose structure when allowed to act on reducing partial starch hydrolysates. As a result, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, produce a novel thermostable non-reducing saccharide-forming enzyme which forms a non-reducing saccharide having a trehalose structure as an end unit when allowed to act on reducing partial starch hydrolysates and also is stable up to a temperature of about 85.degree. C., and found that the non-reducing saccharide can be readily prepared at the objective temperature of over 55.degree. C. when the thermostable enzyme is allowed to act on reducing partial starch hydrolysates. The present inventors also found that trehalose is readily preparable by first allowing the thermostable enzyme to act on reducing partial starch hydrolysates, then subjecting the resultant non-reducing saccharides to the action of glucoamylase or .alpha.-glucosidase. Thus, the present inventors accomplished this invention. Also, the present inventors established preparations of compositions such as food products, cosmetics and pharmaceuticals which contain the present non-reducing saccharides, relatively-low reducing saccharides containing the same and/or trehalose, and accomplished this invention.

Detailed Description Text - DETX (5):

Now, the present inventors found that microorganisms of the genus Sulfolobus, named as "Sulfolobus acidocaldarius" ATCC 33909 and ATCC 49426, and as "Sulfolobus solfataricus" ATCC 35091 and ATCC 35092, are capable of producing a novel thermostable non-reducing saccharide-forming enzyme.

Detailed Description Text - DETX (6):

In addition to the above-mentioned microorganisms, other strains of the genus Sulfolobus and their mutants can be suitably used in the present invention as long as they produce the present thermostable non-reducing saccharide-forming enzyme which forms the non-reducing saccharide having a trehalose structure as an end unit when allowed to act on reducing partial starch hydrolysates.

Detailed Description Text - DETX (51):

Preparation of thermostable non-reducing saccharide-forming enzyme from Sulfolobus acidocaldarius ATCC 33909

Detailed Description Text - DETX (84):

Preparation of thermostable non-reducing saccharide-forming enzyme from other microorganisms of the genus Sulfolobus

Detailed Description Text - DETX (86):

In accordance with the method in Experiment 2-1, non-reducing saccharides were prepared by using these partially purified enzyme preparations, and

studied on their structures to find that, similarly as the thermostable non-reducing saccharide-forming enzyme from Sulfolobus acidocaldarius (ATCC 33909), every enzyme preparation formed non-reducing saccharides having a trehalose structure as an end unit and a degree of glucose polymerization of 3 or higher when allowed to act on reducing partial starch hydrolysates having a degree of glucose polymerization of 3 or higher.

Detailed Description Text - DETX (89):

A seed culture of Sulfolobus acidocaldarius (ATCC 33909) was inoculated in a nutrient culture medium and incubated by a fermenter for about 42 hours in accordance with the method in Experiment 1. After completion of the incubation, the resultant culture was concentrated with an SF-membrane and centrifuged to recover cells. The cells thus obtained were disrupted with ultrasonic, and from the suspension an supernatant was prepared, salted out with ammonium sulfate, dialyzed, and subjected to an ion-exchange column and hydrophobic column chromatography to obtain an enzyme solution containing 18.0 units/ml of a partially purified enzyme preparation having a specific activity of about 20 units/mg protein. The suspension of potato starch having a concentration of 6 w/v % was gelatinized by heating, adjusted to pH 4.5 and 50.degree. C., admixed with 2,500 units/g starch of isoamylase commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and subjected to an enzymatic reaction for 20 hours. The resultant mixture was adjusted to pH 6.5, autoclaved at 120.degree. C. for 10 min, cooled to 60.degree. C., admixed with 30 units/g starch of "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, and subjected to an enzymatic reaction for 24 hours. The reaction mixture was autoclaved at 120.degree. C. for 20 min, cooled to 65.degree. C., adjusted to pH 5.5, admixed with one unit/g starch of the above thermostable non-reducing saccharide-forming enzyme, and subjected to an enzymatic reaction for 96 hours. The resultant mixture was kept at 97.degree. C. for 30 min, cooled and filtered. The resultant filtrate was in usual manner decolored with an activated charcoal, and purified by desalting it with ion-exchange resins in H- and OH-form. The resultant solution was concentrated into a syrup with a concentration of about 70 w/v % in a yield of about 90%. The product exhibits a DE 24.6, and contains as a non-reducing saccharide 12.0% .alpha.-glucosyl trehalose, 5.5% .alpha.-maltosyl trehalose, 29.9% .alpha.-maltotriosyl trehalose, 1.5% maltotetraosyl trehalose and 2.2% .alpha.-maltopentaosyl trehalose, d.s.b. The product has a mild and high-quality sweetness, as well as an adequate viscosity and moisture-retaining ability, and these properties render it arbitrarily useful in food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer and filler.

Detailed Description Text - DETX (99):

A seed culture of Sulfolobus solfataricus (ATCC 35091) was inoculated in a nutrient culture medium and incubated by a fermenter for about 42 hours in accordance with the method in Experiment 3. After completion of the incubation, the resultant culture was concentrated with an SF-membrane and centrifuged to recover cells, which were then disrupted with ultrasonic to obtain an supernatant. The resultant supernatant was salted out with ammonium sulfate, dialyzed, and subjected to an ion-exchange column and hydrophobic column chromatography to obtain an enzyme solution containing 19.0 units/ml of a partially purified enzyme preparation having a specific activity of about 18 units/mg protein. In accordance with the method in Example A-3, the suspension of potato starch having a concentration of 30% was treated with "TERMAMYL 60L", .alpha.-amylase commercialized by Novo Industri A/S, Copenhagen, Denmark, a maltotetraose forming amylase commercialized by Hayashibara Biochemical

Laboratories, Inc., Okayama, Japan, and ".alpha.-amylase 2A", .alpha.-amylase commercialized by Ueda Chemical Co., Tokyo, Japan, and autoclaved at 120.degree. C., cooled to 65.degree. C., admixed with 2 units/g starch of the above thermostable non-reducing saccharide-forming enzyme, and subjected to an enzymatic reaction for 64 hours. The resultant mixture was kept at 97.degree. C. for 30 min to inactivate the remaining enzyme. In accordance with the method in Example A-5, the resultant solution was subjected to the action of "GLUCOZYME", glucoamylase commercialized by Nagase Biochemicals, Ltd., decolored, desalted and concentrated into an about 60% solution. The saccharide solution thus obtained contained about 23% trehalose, d.s.b. In accordance with the method in Example A-5, the saccharide solution was fractionated on column chromatography using a strongly-acidic action-exchange resin to obtain fractions rich in trehalose. The fractions containing about 95% trehalose, d.s.b., were pooled, placed in a vessel and boiled down under a reduced pressure into a syrup with a moisture content of about 4.0%. The syrup was placed in a crystallizer and admixed with one % of anhydrous crystalline trehalose, as a seed crystal, with respect to the syrup, d.s.b., followed by crystallizing the syrup at 95.degree. C. for 5 min while stirring. The resultant was transferred to an aluminum container and aged at 100.degree. C. for 6 hours to form a block. The resultant block was pulverized by a cutting machine and subjected to a fluidized-bed drying to obtain a powdery anhydrous crystalline trehalose with a moisture content of about 0.3 w/w %. The product can be arbitrarily used in hydrous matters such as food products, cosmetics and pharmaceuticals, and their material and intermediates as a desiccant, as well as a white powdery sweetener with a high-quality and mild sweetness.

US-PAT-NO: 5681826

DOCUMENT-IDENTIFIER: US 5681826 A

TITLE: Saccharide composition with reduced reducibility, and preparation and uses thereof

DATE-ISSUED: October 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shibuya; Takashi	Okayama	N/A	N/A	JP
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APPL-NO: 08/ 492691

DATE FILED: June 20, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	6-180393	June 27, 1994

US-CL-CURRENT: 514/54, 536/123.1

ABSTRACT:

A saccharide composition with a reduced reducibility which is prepared by hydrogenating a saccharide mixture comprising reducing saccharides and non-reducing saccharides consisting of trehalose and/or saccharides having a trehalose structure. The saccharide composition has a satisfactory sweetness, taste and stability, and is substantially free from reducibility, so that it can be freely used in a variety of compositions such as foods, cosmetics and pharmaceuticals which are susceptible to reduction.

19 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Detailed Description Text - DETX (2):

The non-reducing saccharide-forming enzymes usable in the present invention include those which can form .alpha.-glycosyltrehalose from one or more reducing amylaceous partial starch hydrolysates selected from those with a glucose polymerization degree of 3 or more which are contained in liquefied starch solutions with a relatively-low DE. Examples of such enzymes are those derived from microorganisms of the genera Rhizobium, Arthrobacter, Brevibacterium, Flavobacterium, Micrococcus, Curtobacterium, Mycobacterium and Terrabacter as disclosed in Japanese Patent Application No.349,216/93. If necessary, thermostable non-reducing saccharide-forming enzymes can be arbitrarily used in the present invention. For example, a thermostable non-reducing saccharide-forming enzyme derived from a microorganism of the genus Sulfolobus as disclosed in Japanese Patent Application No.166,011/94,

titled "Thermostable non-reducing saccharide-forming enzyme, and its preparation and uses", applied for by the present applicant on the day of Jun. 24, 1994, can be arbitrarily used. Any enzyme, which specifically hydrolyzes the linkage between a trehalose moiety and others in alpha.-glycosyltrehalose formed by allowing a non-reducing saccharide-forming enzyme to act on a liquefied starch solution, can be used as a trehalose-releasing enzyme in the present invention: For example, those derived from microorganisms of the genera Rhizobium, Arthrobacter, Brevibacterium and Micrococcus as disclosed in Japanese Patent Application No.79,291/94 can be arbitrarily used. If necessary, thermostable trehalose-releasing enzymes such as that derived from a microorganism of the genus Sulfolobus as disclosed in Japanese Patent Application No.166,126/94, applied for by the present applicant on the day of Jun. 25, 1994, can be arbitrarily used in the present invention.